FOOD AND DRUG ADMINISTRATION

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MEDICAL DEVICES ADVISORY COMMITTEE

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GENERAL AND PLASTIC SURGERY DEVICES PANEL

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57TH MEETING - AFTERNOON SESSION

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MONDAY,

MAY 8, 2000

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The panel met at 1:00 p.m. in Salons F and G of the Gaithersburg Marriott Washingtonian Center, 9751 Washingtonian Boulevard, Gaithersburg, Maryland, Dr. Thomas V. Whalen, Panel Chair, presiding.

PRESENT:

THOMAS V. WHALEN, M.D., Panel Chair
JOSEPH V. BOYKIN, JR., M.D., Voting Member
MAXINE F. BRINKMAN, R.N., Consumer Representative
PHYLLIS CHANG, M.D., Voting Member
DAVID L. DeMETS, Ph.D., Voting Member
SUSAN GALANDIUK, M.D., Voting Member
SALLY L. MAHER, Esquire, Industry Representative
ROBERT L. McCAULEY, M.D., Voting Member
STEVEN I. REGER, Ph.D., Temporary Voting Member
DAVID KRAUSE, Ph.D., Executive Secretary

PRESENT: (CONT.)

APPLICANT REPRESENTATIVES:

VINCENT FALANGA, M.D.
JAY HERSON, Ph.D.
MATHIAS HUKKELHOVEN, Ph.D.
MICHAEL L. SABOLINSKI, M.D.

FDA REPRESENTATIVES:

CHARLES N. DURFOR, Ph.D.
ROXI HORBOWYJ, M.D.
PHYLLIS M. SILVERMAN, M.S.
CELIA WITTEN, Ph.D., M.D.

OPEN PUBLIC:

LAWRENCE B. HARKLESS, D.P.M.

I-N-D-E-X

P-R-O-C-E-E-D-I-N-G-S

(1:27 p.m.)

CHAIRMAN WHALEN: Good afternoon. I am Dr. Thomas Whalen. I apologize for the delay. We are going to get underway. Those of you who attended this morning's session know that we had three members from the morning who are not with us presently. We do have one member who is new for the afternoon. And I would like to ask Dr. Reger to introduce himself.

DR. REGER: I am Steven Reger. I come from the Cleveland Clinic. I am a biomedical engineer, member of the Department of Physical Medicine and Rehabilitation, as well I have a partial appointment in plastic surgery and in biomedical engineering. I am a temporary voting member.

CHAIRMAN WHALEN: Thank you, Dr. Reger.

We will begin the panel afternoon session with an open public hearing. I would like to remind anyone who addresses the panel at this time to please speak clearly into the microphone as, again, the transcriptionist is dependent upon this means to provide an accurate record of the meeting.

As before, we request that anyone making statements at this time disclose whether or not they have financial interests in any medical device company. Before making the presentation to the panel, in addition to stating name and affiliation, state the nature of your financial interest, if any, and whether any of your travel expenses or accommodations have been paid for by someone other than yourself.

We have one scheduled speaker to begin the afternoon: Dr. Harkless. Is Dr. Harkless present? Dr. Harkless, you have five minutes to address the panel.

DR. HARKLESS: Thanks for allowing me to address the panel.

I am Dr. Lawrence Harkless. I am a professor in the Department of Orthopaedics at the University of Texas Health Science Center and the Louis T. Bogy Professor of Podiatric Medicine in Surgery.

I do own 125 shares of Organogenesis stock, which was bought in February of 1998 in a retirement account. And I pay for my own travel.

And, again, thanks for allowing me to address the panel.

Basically I have been working with the diabetic foot for about 25 years of my career. And it is a major disease or problem. Among the 16 to 18 million people in the United States with diabetes, approximately 15 percent will develop a foot ulcer. And the complications of foot ulcer is the most frequent cause of hospitalization among patients with diabetes.

Foot ulcers are a major predictor of future lower extremity amputation in patients with diabetes. Despite the U.S. Public Health Service Healthy People 2000 goal to decrease the lower extremity amputations by 40 percent the last decade, amputations in diabetes actually increased.

As an abstract at the annual meeting of the American Diabetes Association in 1999, the Lewin Group demonstrated in a five percent sampling of the Medicare database in 1995 and '96 that the diabetic foot ulcer prevalence was 7 percent and that particular cost was $1.5 billion. So that's

devastating. And it's the first information that we have looking at a population and the real, real cost of the devastating aspects of diabetic foot ulcers.

Understand the process for pathogenic mechanism. Neuropathy, deformity, limited joint mobility, infection, and vascular disease leading to ulcerations is critical in the management of this particular disease process.

Educational efforts need to be directed toward physicians, health care providers, and a multi-disciplinary team for prevention, early detection, and prompt treatment of diabetic foot ulcers.

Unfortunately, current treatment practices often result in a large percentage of non-healing ulcers with a relapse rate between 40 and 70 percent.

Increased educational efforts and new therapies, such as Apligraf, can lead to a greater number of ulcerations being healed in a shorter time period, decreasing infection and reducing the number of amputations.

Our group was involved in a multi-center

trial with Apligraf. And our experience supports the fact that Apligraf is safe and has provided us with another modality to utilize in our armamentarium to heal diabetic foot ulcers faster.

We support the product, and we hope that the panel will agree that it is something that we should consider utilizing and approve it today.

Thank you for allowing me to present before the panel. Again, my name is Lawrence Harkless from San Antonio.

CHAIRMAN WHALEN: Thank you, Dr. Harkless.

While you're still at the podium, is there any member of the panel with any questions for Dr. Harkless?

(No response.)

CHAIRMAN WHALEN: Thank you, sir.

DR. HARKLESS: Thank you, sir.

CHAIRMAN WHALEN: Is there anyone else from among the public who wishes to address the panel at this time?

(No response.)

CHAIRMAN WHALEN: Thank you. Seeing none,

we'll proceed to the review of the second of the day pre-market approval application. I would like to remind the public observers at the meeting that while this portion of the meeting is open to your public observation, public attendees may not participate except at the specific request of the panel.

We will now begin with the sponsor, Organogenesis Incorporated, with their presentation to the panel.

DR. HUKKELHOVEN: Dr. Whalen, Dr. Witten, members of the Advisory Committee, FDA, and guests, good afternoon. I am Mat Hukkelhoven, and I am Vice President and head of Drug Regulatory Affairs for Novartis Pharmaceuticals Corporation.

This afternoon we will review the efficacy and safety of Apligraf, a unique bi-layered viable skin construct for the treatment of diabetic foot ulcers.

Apligraf as approved in May 1998 by the FDA for the treatment of venous leg ulcers. I can go on because I will talk a little bit without the need for a slide.

The developer of this product and sponsor of this supplemental PMA is the company Organogenesis. Apligraf is manufactured by Organogenesis, and Novartis is the distributor.

Outside of the United States, Novartis is responsible for both registration and distribution of Apligraf. Together our two companies are further investigating the use of this product in clinical trials for other important wound-healing indications.

The already approved indication for Apligraf is shown in this slide. Specifically, as you can see, Apligraf is currently indicated for use with standard therapeutic compression, for the treatment of noninfected partial and full-thickness skin ulcers due to venous insufficiency of greater than one month duration and which have not adequately responded to conventional ulcer therapy.

The use we are now seeking for Apligraf is that Apligraf would also be indicated for the treatment of full-thickness neuropathic diabetic foot ulcers of greater than two weeks duration that extend through the dermis but without tendon, muscle,

capsule, or bone exposure.

Although Apligraf as a viable bi-layered skin construct is a unique approach to treating chronic wounds, Apligraf has already been applied extensively both in clinical studies as well as in commercial use.

Up until now, Apligraf has been used in approximately 1,000 patients in various clinical trying settings. These include the 161 Apligraf-treated patients in the venous leg ulcer study; the 112 patients in the diabetic foot ulcer study, which we are going to review today; as well as further exposures from studies in epidermolysis, bullosa burn, donor site, and excisional surgery.

Approximately 560 patients have so far been exposed to Apligraf in various post-marketing settings in the U.S. and in Canada. Since its approval in Canada and the U.S., the estimated commercial use of Apligraf has been in over 10,000 patients.

Next slide, please. Today, as mentioned, the sponsor is seeking approval for Apligraf for the

treatment of diabetic foot ulcers. The data which will be presented to you will demonstrate that Apligraf provides an effective and safe treatment for patients with neuropathic diabetic foot ulcers.

The presentation today will begin with Dr. Vince Falanga, Professor and Chairman of the Department of Dermatology and Skin Surgery at Roger Williams Medical Center and Professor of Dermatology of Boston University School of Medicine.

Dr. Falanga will talk about the impact of diabetic foot ulcers and their pathogenesis. He also participated in the pivotal diabetic foot ulcer study.

Dr. Falanga will be followed by Dr. Michael Sabolinski, who is the Senior Vice President of Medical and Regulatory Affairs at Organogenesis. Dr. Sabolinski will review in detail the results of the pivotal study comparing the efficacy and safety of Apligraf plus standard care to standard care alone in patients with neuropathic diabetic foot ulcers. In his presentation, Dr. Sabolinski will also address some of the FDA questions which are asked to the panel.

Joining us today, in addition to Dr. Vince Falanga, we have two other investigators who have participated in the diabetic foot ulcer study. Dr. Aristidis Veves is Research Director at the Joslin Beth Israel Deaconess Foot Center and Microcirculation Lab and instructor in medicine at Harvard Medical School.

Dr. Elliot Chaiket is Associate Professor of Surgery, Division of Vascular Surgery of the School of Medicine, at Emory University, Atlanta, Georgia.

I would now like to turn the podium to Dr. Falanga, who will review the impact of diabetic foot ulcers and their pathogenesis.

DR. FALANGA: Thank you very much and good afternoon.

I'd like in my presentation today to outline the problem of diabetic ulcers. I'd like to also discuss issues related to the treatment and pathophysiology of these ulcerations.

And then I'd like to introduce the device that has been discussed today, Apligraf, in terms of what it looks like and some photographs outlining some

of its features. And, finally, I should provide some actual data regarding how this construct behaves *in vitro*.

The problems with diabetic foot ulcers, of course, is important because there are 16 to 18 million people in the U.S. who have diabetes. And of these, about 15 to 20 percent; that is, about 3 or 4 million ulcers, will develop in these patients during their lifetime.

Patients who have diabetes are 15 times more likely to undergo an amputation following injury. And this results in about 67,000 or so lower extremity amputations per year in the U.S. The costs to our society are staggering. It exceeds $1 billion.

Next. There isn't necessarily any causal relationship between amputation and death and other complications, but certainly the associations are strong. After one major lower extremity amputation, the 3-year survival rate is 50 percent, the five-year survival rate is 40 percent.

And also contralateral amputation will occur in 42 percent of patients once in 3 years after

the first amputation and in 56 percent of patients within 3 to 5 years after the first amputation. Of course, these figures are frightening to our patients.

Next. The etiology of diabetic foot ulceration relies on three fundamental abnormalities and a fundamental triad: Neuropathy, vascular insufficiency infection: neuropathy comprising sensory, motor, autonomic dysfunction; vascular insufficiency involving the distal vessels and complicated by poor collaterals and medial calcinosis.

And, of course, we know that patients with diabetes appear to be particularly prone to infection. They certainly have an increased risk for infection. They seem to have defective host response. And, actually, they may have systemic signs and symptoms which are absent and complicate the problem.

In all of this, minor trauma appears to play a major role. And patients who have an insensate foot are not able to adjust accordingly when an ulcer develops.

Next. The failure to off-load leads to high plantar foot pressure. So that there is a role

in ongoing mechanical trauma. There are shearing forces and, of course, biomechanical dysfunction, which is complicated by the enteropathy-induced muscle imbalance. So that certain portions of the foot are exposed to excessive pressures. And it's these excessive pressures that lead to ulceration in the insensate foot.

Next. The benefits of healing a diabetic foot ulcer are self-evident, but they are nicely categorized in a recent meeting called the Consensus Development Conference on Diabetic Foot Wound Care, which was organized by the American Diabetes Association. The aims are to control infection, maintain health status, prevent amputation, improve function and quality of life, and reduce costs.

Next. There are certain features of the treatment that have become standard in the treatment of diabetic foot ulcers. These are extensive debridement of nonviable tissue. Saline-moistened dressings are commonly applied as the primary dressing.

And, of course, off-loading to decrease

pressure on the extremity is critical. Treatment of infection is essential. And, of course, patients who have arterial insufficiency may need arterial reconstruction.

Next. This is a recent publication that will also be mentioned later on by Dr. Sabolinski. It's the healing of diabetic neuropathic foot ulcers receiving standard treatment.

This is a meta analysis that was recently published in "Diabetes Care" by Dr. Margolis and colleagues from the University of Pennsylvania. It involved the systematic review of the control groups of ten randomized clinical trials.

The endpoint of these trials was complete wound closure. And there were 6 control groups available which looked at complete wound closure at 20 weeks and 4 control groups that looked at complete wound closure at 12 weeks. And when you look at the rates of healing, complete closure was achieved in 31 and 24 percent, at 20 weeks and 12 weeks, respectively.

Next. This brings out the problem of: Is

there something other than off-loading that may be wrong with diabetic wound care? And one question that has been bouncing around for a long time and which remains somewhat controversial is whether there is indeed a failure to heal in diabetic patients.

Certainly we have discussed the underlying pathophysiology of ischemia, neuropathy, and infection. There has been some work suggesting that there is impaired wound healing in diabetic patients. More recently, there has been increasing interest in looking at some of the histological markers and looking at the progress through the wound-healing process in patients with diabetes.

For example, abnormal blood vessels of the wound edge and base of diabetic ulcers have been found. These are often cuffed with several extracellular matrix proteins, including laminin, collagen, fibronectin, and fibrin. And some of these they can actually bind other extracellular matrix proteins and cytokines. So this may play a part in the pathogenesis.

Another piece of evidence that appears to

be emerging is that certain extracellular matrix proteins are deposited or remain for an extended period of time in patients with diabetic ulcers.

One recent publication looked at fibronectin, chondroitin sulfate, and tenascin. I'd like to show you an example from this publication, which was published in 1998.

Next. This is prolonged expression of fibronectin in diabetic ulcers. The same thing was shown for chondroitin sulfate and tenascin. A and D are the normal wound-healing process without wounding, after wounding, at 19 days, at 3 months, and at 4 months. As you can see, fibronectin increases, reaches a peak, and then remodeling occurs and it disappears.

It looks like in patients with diabetic foot ulcers when you look histologically, there's a persistence to certain extracellular matrix proteins. And the point has been made that these wounds do not heal. They're stuck in a certain phase of the wound-healing process. They're unable to come out of it.

So certainly there could be things that could be used to stimulate these wounds to heal. There's been some work related to pressure-induced injury, for example, that has stated that cells in the epithelization margin here actually are senescent. So there may be room here for a stimulatory effect of agents to allow these ulcers to heal more properly. I'd like to discuss Apligraf at this point.

Next. As mentioned, Apligraf is a viable bi-layered skin construct consisting of an epidermal layer formed by human keratinocytes with a well-differentiated stratum corneum, a dermal layer composed of human fibroblasts in a bovine Type 1 collagen lattice. And there are matrix proteins and cytokines in Apligraf which are very similar in distribution to those found in human skin.

Importantly, Apligraf does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels, or hair follicles, and certainly does not contain certain professional antigen-presenting cells.

I'd like to mention that we do not know

the mechanisms of action of binds in new skin and particularly Apligraf. There are some findings that are related to *in vitro* work. I'd like to discuss those with you next.

First, this is Apligraf, what it looks like. This is the piece of Apligraf that has been picked up. This is the plate. This is the transwell in which it sits. This pink material is the agar on which the Apligraf sits and which provides nutrients for the construct.

What I'm going to show you in the next slide is really just an example of the fact that it's a very elastic type of device and many things can be done *in vitro* or *in vivo* and can be manipulated very easily.

Next. This is just an example. This is me actually picking up the construct from its transwell. And, as you can see, it can be easily handled and manipulated. And it's a very easy device to handle.

Next. Histologically, it looks remarkably similar to human skin. This is Apligraf. This is

human skin, the epidermis, the dermis. There are some differences, of course. You do not have this undulating epidermis in Apligraf. And, of course, the cellular components of the dermis are on the fibroblasts, even though this time many extracellular matrix proteins have been deposited.

Next. As I mentioned earlier, we do not know the mechanism of action of Apligraf. This is work done *in vitro* which looks at the cytokine expression in Apligraf, compares it to human skin so that the cytokine profile certainly for these cytokines listed here are identical for Apligraf and human skin. And this is the expression in the two different components, cellular components of Apligraf.

I'd like to just point out that in some situations, for example, if you look at insulin growth factor 1, there's not much produced in keratinocytes or dermal fibroblasts, but when these cells are placed together in a construct, then you do get expression. And this suggests at least that there may be synergism between the two cellular components of Apligraf.

Next. We do not know how the mechanisms

of action of Apligraf and certainly even *in vivo*, there are some questions related to what you are observing at any particular time.

These are just examples that I've taken Apligraf in place over a diabetic ulcer one week later. This is a venous ulcer that's healing with Apligraf. And there is massive re-epithelialization here occurring two weeks after the application.

Most importantly, I'd like to point out a stimulation that might occur. We don't know it exactly, but it seems to stimulate the edges of the wound to heal.

Here's the Apligraf one week later, and here are the edges of the wound that appear to be activated and ready to migrate towards the center of the wound. So I think it stimulates the wound to heal. And much more work needs to be done to understand these mechanisms of action.

Next. *In vitro*, there are certain things you can do which can address the question of how dynamic this construct is. It is a very dynamic construct.

Here is the Apligraf, the epidermis, the dermal component. You make an injury into it. You cut it. And then this is over a 24-hour period. You can see that, actually, the epidermis migrates and it covers the defect, as you can see here. And, actually, we have a closeup of this, as you can see, but it covers the defect.

You might wonder. *In vivo*, actually, we're putting Apligraf over the dermis. So perhaps a better experiment might be to place the construct after wounding it over a dermal equivalent. And this has been done.

Next. So here is the construct that's been wounded. And now it sits over the dermal equivalent. So basically I have the Apligraf here, the dermal component, the dermal component, and now it's over a dermal equivalent.

In 12 hours, you see there is something already happening. At two days, there is definite migration of the epidermis over the dermal equivalent. And at four days, there is complete re-epithelialization with stratification of formation

of the stratum corneum, as you can see here, suggesting that Apligraf is able to respond to injury.

Next. So, in conclusion, neuropathic foot ulcers have a critical impact on the morbidity of patients with diabetes and the risk for amputation. They are difficult to heal, as shown also by the meta analysis that was recently published.

Even with good standard care, they involve neuropathy, trauma, and continued pressure injury and may be associated with a lack of progression through the normal wound-healing process. As a viable bi-layered skin construct that is also capable of stimulating a healing response, Apligraf may be, therefore, of benefit to patients with diabetic foot ulcers.

At this point, I would like to introduce Dr. Michael Sabolinski, who is the Senior Vice President of Medical and Regulatory Affairs at Organogenesis and will tell us about the trial.

Thank you.

DR. SABOLINSKI: Thank you.

Good afternoon. My talk today will focus

on Protocol 95-DUS-001, a multi-center prospective, randomized, controlled clinical trial of Apligraf for the treatment of diabetic foot ulcers.

Next slide, please. The presentation outline is shown. I'm going to discuss first the study design; then the patient population; efficacy; the sponsor's subgroup analysis; and lead to FDA questions; safety; risk-benefit; and, finally, a conclusion.

Next slide. The objective of our trial was to compare the efficacy and safety of Apligraf therapy plus standard care to standard care alone for the treatment of neuropathic diabetic foot ulcers.

Next. Standard care is extensive debridement of nonviable tissue, saline-moistened dressings, and off-loading to decrease pressure on extremities.

The study time line is as follows. At study day minus seven, preliminary eligibility is determined. At study day minus seven, randomization takes place. And we stratified for two factors, age 18 to 70 years and 71 to 80 years, and charcot status,

either non-present but inactive.

From study day minus seven to day zero, there was a one-week run-in. And at study day zero, final inclusion/exclusion criteria were determined. And at day zero, treatment was initiated.

From day zero to week 12, efficacy was evaluated weekly. And from day zero through month six, safety was evaluated over the entire study.

Next. Our study population was consenting patients with full-thickness foot ulcers of neuropathic etiology without tendon, muscle, or bone exposure.

Next. Key inclusion criteria are shown in this slide: patients with Type I or Type II diabetes, ulcers of at least two weeks duration, full-thickness neuropathic ulcers. Size of the ulcer post-debridement must have been between one and 16 centimeters squared. Dorsalis pedis and posterior tibial pulses were obtained. And glycosonated hemoglobin, hemoglobin A1c, was between 6 and 12 percent. And patients were between the ages of 18 and 80.

Next slide. Key exclusion criteria: clinical infection at the target ulcer site, ulcers with sinus tracts or tunnels, clinical assessment of vascular disease, healing rates which exceeded 30 percent during the one-week run-in prior to day zero. Ulcers on the dorsum of the foot were excluded, as were ulcers on the calcaneus and ulcers with tendon, muscle, capsule, or bone exposures.

Next. Study treatments. Both patients randomized to the Apligraf group received surgical debridement, Apligraf contacting the wound, saline-moistened dressings, and total weight off-loading. Those randomized to the control group received surgical debridement, saline-moistened dressings contacting the wound, and total off-loading.

Next. Regarding the Apligraf treatment group, Apligraf was permitted to be applied one to five applications. All patients randomized to the Apligraf group had Apligraf applied at day zero.

Additional applications were permitted at weekly intervals if the wound was less than 100 percent closed and not progressing to healing. And

the last application permitted was at study week four.

Next. Supported therapies for both groups: extensive debridement to remove infected tissue and to expose the true size of the wound, effective off-loading. Crutches or wheelchairs were required for the first six weeks of the study in both groups, custom pressure-relieving footwear, custom tri-density sandals for at least four weeks post-closure.

Next slide. Now I'll move on to patient populations. This study had 24 centers that enrolled patients. Two hundred seventy-seven patients were randomized. Two hundred eight patients were treated: 112 Apligraf patients and 96 control.

Next slide. The following three or four slides show characteristics of the treated population. There were 112 Apligraf, 96 control. And this slide shows demographic characteristics of age, gender, race, and type of diabetes. Apligraf and control are comparable for the four factors shown on this slide.

Next. Demographic characteristics are continued: height, weight, body mass index. And,

again, both groups are clinically comparable.

Next. Baseline ulcer characteristics, wound area, wound duration, ulcer location, that were captured by at the toes, at the metatarsal head and mid-foot. And number of ulcers on the study foot again were comparable between groups.

Next slide. And, finally, other baseline characteristics, glycosonated hemoglobin, a smoking history, currently smoking, and systemic antibiotics used within 30 days were comparable between groups.

Next. The patient disposition in the study: 112 Apligraf patients were treated, 96 control. In both groups, over 80 percent of the patients completed the 12-week efficacy period. And over 75 percent of the patients completed the full 6 months of the study. Both groups are comparable.

Next. The reasons for discontinuation are shown. There are a total of 22 Apligraf patients and 22 control patients, who are discontinued over the 6 months of the study. The reasons are shown. And there are no remarkable differences between groups.

Next slide. Regarding our stratification

for age, 18 to 70, 91 percent of the total population treated were between the ages of 18 and 70, and 9 percent of the patients were between 71 and 80. And there are some numerical differences, but they are statistically comparable.

Next. In the subgroup population, the distribution of Charcot joint deformity, no Charcot occurred in 81 percent, Charcot occurred in 19 percent. And, again, there are some numerical differences, but there is a comparability between groups.

Next. The efficacy portion of the study. The primary efficacy endpoint is complete wound closure by week 12. And wound closure is defined as full epithelization of the wound with the absence of drainage. And epithelization is defined as a layer of epithelium visible on the wound surface. This is the Wound Healing Society's definition that was applied.

Next. The assessment of wound closure. First, investigators were required to complete the following. And this is a direct quotation from our case report form, "Complete wound closure: Yes or No.

Complete wound closure will be dignified as full epithelization of the wound with the absence of drainage."

Additionally, open wound tracings were performed by the investigators. Wound areas were determined by a masked third party using computerized planimetry and were used to confirm investigator assessment of complete wound closure. And, finally, photographs were taken at each visit but were not used to assess wound closure.

Next. The primary efficacy endpoint statistical analyses. The incidence of 100 percent wound closure by week 12, we applied the Fisher's exact two-tailed test and the Cochran-Mantel-Haenszel test, which adjusts for center.

The second analysis, incidence of 100 percent wound closure per unit time by week 12, there were 2 timed to event analyses performed: a Kaplan-Meier life table, which is an unadjusted analysis and accounts for all data in the study over the 12-week efficacy period; and the Cox's proportional hazards regression, which is an analysis

that adjusts for risk factors.

Next. The frequency of complete wound closure is shown in this slide. Sixty-three of the 112 Apligraf patients attained wound closure at a rate of 56 percent. Thirty-six of 96 of the controlled patients attained wound closure at a rate of 38 percent. The *p* is less than .05. The Cochran-Mantel-Haenszel test, which adjusted for center, also showed a *p* of less than .05.

Next. The time to complete wound closure using the Kaplan-Meier life table analysis. This slide shows that Apligraf had a median time of complete wound closure of 65 days compared to control of 90 days, *p* less than .05. And the median time is defined as when 50 percent of the patients attained complete wound closure by the Kaplan-Meier life table analysis. The estimated frequency of complete wound closure at week 12, specifically day 84, is Apligraf 56 percent and control 39 percent.

Next. In the final analysis is the Cox's proportional hazards regression. And this is done in order to test whether risk factors may have been

distributed unevenly between groups and could have impacted on the unadjusted data.

We see all of the factors that were prospectively stated and entered into the Cox's regression analysis listed, some: ulcer duration, location, number of ulcers, age, smoking, nutritional status, glucose control. Those that are asterisked are those that ended up as being statistically significant in the final model.

Next. The results of the Cox's proportional hazards regression showed an Apligraf treatment effect with a risk ratio of 1.59 and a 95 percent confidence interval of 1.261 to 1.996, with a *p* less than .05. This risk ratio means that Apligraf over the 12-week period of observation increased the probability of healing over control by 59 percent.

The estimated frequency of complete wound closure at week 12, day 84, with the Cox's proportional hazards regression model is Apligraf 58 percent and control 32 percent.

Now we move to the durability of response, which means: Of those patients that demonstrated

complete wound closure, how many in each group had complete wound closure for greater than or equal to four weeks?

In the Apligraf group, this was 52 of 63 patients, or 83 percent. In control, it was 31 of 36, 86 percent. And the *p* is greater than .05.

Next. Ulcer recurrence is shown in this slide. Of the 63 and the 36 patients who had complete wound closure evaluated by study week 12, at month 4, 7 Apligraf patients recurred and 3 control recurred. At month five, one patient in each group reopened and at month six, three in the Apligraf and four in the control. The *p* is greater than .05.

Next. The summary of our results and efficacy. When compared to control, Apligraf improved the frequency of complete wound closure 56 percent compared to 38 percent, reduced the time to complete wound closure 65 days compared to 90 days, and increased the probability of healing by 59 percent over 12 weeks. These were all statistically significant findings. And Apligraf showed a comparable incidence of recurrence when compared to

control.

Next. Now, the sponsor performed analyses in subgroups. And I'm going to use this as an opportunity to answer some of the FDA questions, both to us and that were posed to the Advisory Panel.

First I'd like to make some general comments just regarding how we approach subgroups in the design of the study and how they were used.

Next. Our overview is that the purpose of our trial was to determine the effectiveness of the Apligraf in the overall target population of neuropathic diabetic foot ulcers and not in individual subgroups.

The subgroup analyses were needed to identify possible candidate risk factors for Cox's proportional hazards analysis. And Cox's analysis was used to adjust for risk factors that may have contributed to the overall conclusions. After adjusting for risk factors, the unadjusted and adjusted data were compared.

Next. Now, this slide shows all of the co-variates and shows all of the subgroups. There are

at least two groups per factor. Some, for instance, are entered as continuous variables. And all of this represents about 30 subgroups that we considered in our analysis.

Next. When we compare the unadjusted data to the adjusted data, we show that, even when taking into account those 32 groupings, the Apligraf treatment effect remained. The estimated frequency of complete wound closure for the Cox analysis was 58 percent and 32 percent, Apligraf and control. And the unadjusted frequency by comparison was 56 Apligraf and 38 percent control. So our conclusion is that the differences between Apligraf and control were not due to an imbalance in risk factors.

Next. Now, specific to the Charcot joint deformity subpopulation, this subgroup -- again, the purpose of our trial was to determine the effectiveness of Apligraf in the overall population of neuropathic diabetic foot ulcers. I think the issue is appropriately brought up about Charcot subgroup.

The Charcot subgroup was included in our study because these patients are a part of the real

life neuropathic diabetic foot ulcer population. Stratification in our study was performed to help ensure balance between the Apligraf and control groups.

The study was not meant to be powered to show significance in this small group, which only makes up between about 10 to 20 percent of the overall population.

Next. Any explanation for the apparent differences of Apligraf and control. For instance, we stated in our PMA the difficulty in immobilization or off-loading or ulcer area or ulcer duration we believe remains speculative in this small subgroup.

Next. Now I'm going to show you three or four slides to illustrate the following points. First, the frequency of complete wound closure between the Apligraf and control group was not statistically significant.

Second, the Apligraf and control groups for those patients with Charcot joint deformity were not comparable for baseline characteristics.

Third, the ulcer area in Charcot patients

subgroup is larger than in the overall population for both groups. And, finally, the ulcer area of the healed Apligraf patients was larger when compared to the control group.

Next. This slide shows the distribution which I put up previously. And it's just to remind you that there was a total of 81 percent that had no Charcot in our study and 19 percent that did have Charcot, 17 Apligraf and 22 control patients.

Next. Now, in this slide, there are a number of comparisons that can be made. These show patients both with no Charcot and Charcot. Reading across on the top line, we can make inter-group comparisons, Apligraf to control. Sixty-three percent of the Apligraf patients with no Charcot healed, compared to 38 percent, *p* less than .05.

In reading across from this slide for Charcot, 18 percent of Apligraf, 36 percent of control healed, *p* of .29. Intra-group comparisons can also be made.

First I'll start with the control group. Here we see that no Charcot compared to Charcot heals

38 percent and 36 percent. There have been publications saying that Charcot poses a twelve-fold increased risk or negative factor for healing. These results are surprising; in the Apligraf group, 63 percent compared to 18 percent. And I'd like to show you some characteristics that I think can help explain these differences.

Next. There is a series of slides where numbers are in a different color. And they're meant to draw your attention to them. This shows baseline ulcer characteristics: wound area, wound duration, and ulcer location. This is the Apligraf group and control who have Charcot joint deformity, the 17 Apligraf and 22 control.

Apligraf, both the mean and median ulcer duration, is higher than in control. In fact, looking at the mean, it's 24 months, or 2 years, compared to 5 months. That's a significant difference.

Next. Looking at this slide, we can make both inter and intra-group comparisons. First, for both Apligraf and Charcot, we see all patients treated in Charcot. The Charcot ulcer size is larger when

compared to the entire population in both the Apligraf and control groups.

In the Apligraf group, the median duration of the Charcot patients is greater than for all patients. And, again, it's 24 months median in the Charcot Apligraf group and for all patients is 6 months. That's something that perhaps could have affected the 60 and the 18 percent difference between the 2 groups.

And, finally, the location of the ulcers. We see that there is a distribution by anatomical location. And three patients in each group had ulcers occurring at either metatarsal heads or toes.

Next. The baseline ulcer characteristics of the healed patients with Charcot, I've highlighted the ulcer areas. Three patients healed. They were three patients with large ulcer areas.

In control, if we look at the response rate in the control group of 38 percent for all patients and 37 percent for Charcot patients, the demographic or the characteristic of size, the control patients who had healing in Charcot group were smaller

ulcers. The Apligraf, all three were larger ulcers. And, again, the location of those that healed was distributed between groups at places other than mid-foot.

Next. So with Charcot, we do believe that any explanation for apparent differences between Apligraf and control remain speculative in the small subgroup.

Next. Finally, study location. This slide shows the results by anatomical location. First, the large group of patients with their ulcers occurring on the metatarsal head; looking across, 62 percent Apligraf compared to 41 percent control. *P* is less than .05.

At the mid-foot, which is the next largest group with a total of 64 of the 208 patients, it's a 40 percent response rate in Apligraf and 24 percent in control. That *p* is greater than .05, but it is at a level of .185. And, finally, at the toes, you see 14 of 22, 64 percent Apligraf, 8 of 13 control, 61.5. And that *p* is equal to one.

Next slide. This slide shows a baseline

ulcer characteristic that certainly isn't surprising but bears pointing out. We look at both the Apligraf group and the control group, and we list the wound area of ulcers occurring at the toes, metatarsal head, mid-foot. We do this for each group.

There are a lot of numbers on this slide. I'll just go to the median. In the Apligraf group, the median size in millimeters squared is 115 at the toes, 166 at the metatarsal head, and 291 at the mid-foot.

A similar pattern is shown for control, 125, 152, and 269. Smaller ulcers occur at the toes followed in size by metatarsal heads, which are somewhat larger. And then mid-foot showed the largest ulcer.

One other interesting demographic. When we look at the ulcer duration, in the Apligraf group, the median ulcer duration of those ulcers located at the mid-foot was 13.5 months compared to 6 months. Again, when we look at anatomical location and try to break it out, there are a number of factors going on.

Next. So our conclusion is that any

explanation for the apparent differences between Apligraf and control for study ulcer location, whether it's ulcer size, which I showed, or off-loading, or ulcer duration, remains speculative in this small subgroup.

Next. And, finally, there is another category that I wanted to show two things.

Next slide. First, we were asked by FDA -- and I believe the panel received this in your briefing information. There were nine total patients who violated the ulcer size requirement in the protocol: seven Apligraf patients and two control. Six Apligraf patients were too small. And both control patients had ulcers who were too small.

When we take these patients out, the frequency of response in 105 Apligraf and 94 control is 54 percent and 37 percent, *p* less than .05. The median time is 70 days and 90 days. And that also is a *p* less than .05.

Next. This next slide is a completely different thought. And, again, you have this in your briefing book. And we were asked to make comments on

it. It shows the number of applications, 1 through 5, in the 112 Apligraf patients.

This shows the number of patients who received one, two, three, four, and five. And this shows the percentage in the total population. This shows the incidence of wound closure.

So in this slide, we see that 9 percent of the patients receive one application, but 9 of 10 closed, for 90 percent. In those that received five, this was the most common, the majority of patients received five; in fact, 59 of 112, 53 percent of the population. And the closure rate was 46 percent, 27 of 59. And the overall response is shown with Apligraf and control.

It shows that, even though there are larger numbers of patients who heal with four or five applications, there are also larger numbers of patients treated.

Next slide. So our conclusion overall for subgroups, our subgroup shows small patient numbers, generally less than or approximately 20 percent of the treated population and confounding variables,

co-morbidities, demographic characteristics, and some baseline ulcer characteristics. And we get back to our original purpose.

After adjusting for risk factors, the significance of the effectiveness data remained in the overall target population for Apligraf versus control.

Next. Now safety. I'm bringing you back to the entire population and just showing a demographic that was shown in the first four slides. In the Apligraf group, there were 12 patients who had more than one ulcer on the study limb. In the control, there were six. There were 15 patients in the Apligraf group and 10 patients in control who had ulcers not on the study limb.

Next. The next two or three slides show the incidence of the most common adverse events by first occurrence. And we show them in descending order of frequency.

So wound infection, study ulcer occurred in a total of 25 patients in the population. We show Apligraf in this column control and then by a Fisher's exact test, whether the numbers are not significant or

are less than .05.

We show that, for instance, 10.7 percent, 13.5 percent of patients in Apligraf and control had wound infections associated with the study ulcer. 13.4 percent of Apligraf and 7.3 percent had wound infections not associated with the study ulcer.

When we go down the list, none of the events are statistically significant except the small number of patients with osteomyelitis in the control group associated with the study ulcer.

Next. This slide continues on and shows those events that are related to the skin or appendages by our coding system. In your handout, neuropathic. These are new neuropathic ulcers. They were coded as new ulceration. They're neuropathic ulcers; non-study site; and then non-neuropathic ulcerations, which included erosions, fissures, lacerations. We see 17 percent non-study ulcer-related in Apligraf, 9.4 control; non-neuropathic 11.6, 11.5. None of the events shown are statistically significant.

Next. Finally, the incidence of

non-wound-related events, peripheral edema, accidental injury, pain, non-wound infection, diarrhea, hypoglycemia. There's comparability between both groups. The Fisher exact test shows a *p* of less than .05 for peripheral edema and for diarrhea, both occurring at a lower incidence in the Apligraf group.

Next. And, finally, this slide has a lot of information on it. I've shown you the efficacy response by number of applications in the control group. This is meant to sum the total number of adverse events in the Apligraf-treated patients and break it out by the cohort who received one, two, three, four, and five.

And I'll use the first and last line. There were ten patients, nine percent of the population, who received one application. There were a total of 42 of the adverse events shown in the previous slide. And that is a frequency. Forty-two over 345 is 12 percent.

Looking at it another way, there were 4.2 adverse events per patient, 42 occurring in 10. And when we go down, we see that the total number of

adverse events occurs most often in that patient population, the cohort of Apligraf that received 5, but the number of adverse events per patient is 3.2, 184 distributed over 59. I bolded the percentage of the distribution of those that got 5, 53 percent, and those the frequency of the adverse events.

And, finally, the control group and Apligraf group are summed. There were 345 adverse events in 112 patients, at a number of adverse events per patient of 3.1, 329 and 96 patients for 3.4 in control group. Overall, the adverse events are comparable group to group.

Next. And this is just a slide that reminded me to make this point because I was getting confused when I looked at this earlier. While the number of reported adverse events increases as a function of the number of Apligraf applications, the percent of reported adverse events is very similar to the percent of patients receiving one, two, three, four, or five applications. This suggests that no direct correlation exists between the number of Apligraf applications and adverse events.

Next. Another parameter that we show was serious infections at the study ulcer. And this is a regulatory definition where serious means fatal, life-threatening, permanently disabling, or requiring inpatient hospitalization.

Wound infection, cellulitis, osteomyelitis, abscess, gangrene, and fungal infection are shown. There are a total of 12 events in the Apligraf group considered serious at 10.7 percent, 19 in the control, 19.8 percent. They're comparable.

Next. And, finally, this is an analysis that was discussed with FDA last week and I think is useful as a worst-case event. We counted anything that occurred on the study limb. And this is by the number of patients, the total number of patients with reported infection events on the study limb.

And infection is by first occurrence. That would include a wound infection, cellulitis, osteomyelitis, abscess, gangrene, or fungal infection. One patient contributes once. So the first infection on study limb, it occurred in 38 patients of 112 treated patients, for 34 percent in Apligraf. In

control, it was 36 of 96, 38 percent in control.

Next. And some additional safety parameters, the amputations on the study limb. And these are patients, 7 patients in the Apligraf, 15 in the control. Thirty-three Apligraf patients were hospitalized for any reason, 36 patients in the control.

One Apligraf patient and three control patients were admitted with sepsis. And there were two life-threatening events in the control group and one death. None of the life-threatening adverse events and the one death, it was not related to control treatment.

Next. So the summary of our safety results is really captured in the first bullet. Adverse events are comparable between Apligraf and control. Certain parameters were statistically significant, *p* less than .05. Associated with the study ulcer, osteomyelitis was less frequently observed in the Apligraf group.

On the study limb, amputations occurred less frequently in the Apligraf group. And systemic

events, diarrhea and peripheral edema, occurred less frequently in the Apligraf group.

Serious infections are comparable. And additional safety parameters, hospitalization, sepsis, life-threatening adverse events, and death are comparable.

Next slide. I just have a slide or two on risk-benefit.

Next. This slide Dr. Falanga has shown previously. And the Consensus Conference of the American Diabetes Association says: Why is it that you treat diabetic foot ulcers? It's to control infection, to help maintain the overall health status of patients, to prevent amputation, to improve function and quality of life, and to reduce costs.

Next. We conclude from the data in our study observed over a six-month time point for the patient population defined in the protocol Apligraf provided effective treatment and did not pose an increased risk. Apligraf has a favorable risk-benefit ratio compared to standard treatment in patients with neuropathic diabetic foot ulcers.

Next. And, finally, our conclusion.

Next. Apligraf treatment is safe and effective and provides significant benefits for patients with neuropathic diabetic foot ulcers.

Thank you.

CHAIRMAN WHALEN: Thank you.

Perhaps there are questions of the sponsor by panel members. We'll start perhaps with Dr. Chang.

DR. CHANG: Briefly, did you have any breakdown of differences in rate of healing among centers? And was there a statistical difference? And was there any difference in the handling of the ulcers or treatment of protocol to make a difference among the centers?

DR. SABOLINSKI: I'm going to ask for two slides to be shown. I'll answer the question. One, I'd like the slide for our seven pooled centers that show healing. And the other, I'd like the slide for the individual centers broken out.

Our study had 24 centers. And we used an algorithm. In order to test for a center interaction, we used an algorithm of pooling centers. And we ended

up with seven pooled centers.

This was done previously in the venous leg ulcer study, and it's done because some of the centers; in fact, in this study, as you'll see with the slide, eight of them, had very few patients.

And the algorithm was that each center needed to have at least 15 patients. So pooled Center 1 is one individual center. Pooled Center 2 is another individual center. When you get to 3, 4, 5, 6, and 7, you're combining centers.

And the answer to your question is that in each of the centers, pooled centers, Apligraf was superior to control for the frequency of healing.

I don't know the answer for the rate of healing. There was some considerable difference in the absolute heal rate, however. For instance, in Center Number 2, we see that in the Apligraf group, 84.6 percent heal. And in Center Number 7, 40 percent heal. In the control group, we see Center Number 2 healing at 63.6 percent, and Center Number 5 heals at 28.6 percent.

Though the treatment and compliance to the

protocol is comparable, I think the difference between Center Number 2, at least in the control group, is that the median ulcer size I believe was 115 millimeters squared in the control patients at Center Number 6. It was approximately two centimeters squared.

We do see differences. Directionally, the pooled centers are all comparable. We test this statistically using a Breslow-Day test and a Cochran-Mantel-Haenszel test. And we don't see that there is a center by treatment interaction, which means that it didn't matter which pooled center you went to. Apligraf was going to work best.

Now, the slide for individual centers -- and I don't know if they can bring it up right away. When you see all 24, some of the centers who have 5 patients or 6 patients where you might have treated 2 in one group and 3 in another, you'll see differences of 66 percent, for instance, in control, 33 percent in Apligraf. And it's because you might have healed one of three and two of three.

So yes, there were differences in small

numbers. And I think the breakout is that there were eight centers, the small ones that you would have seen a different direction. And I'm having a hard time seeing this. I don't know if it's able to be read by the advisers.

So, for instance, I'm just looking. I'm moving down to -- you really should get the slide of centers. Now, I'm just looking. I don't see a case where Apligraf -- in Center 4, one control patient is treated. That patient healed. And that's 100 percent. Two of the four Apligraf patients were treated, and that's 50 percent. That's a directional difference, and there were eight of those.

But when the centers were combined by pooling in rank order of their patient enrollment and then making sure that each had 15 so that you had a good number to look at, the direction was always consistent.

DR. CHANG: Just a follow-up question.

DR. SABOLINSKI: Sure.

DR. CHANG: So your attestation is it was really the size of the original wound that seemed to

make the difference and not going back or trying to inquire if there was a different management style in following the protocol that resulted in data that didn't fit the trend.

DR. SABOLINSKI: In fact, regarding the management style, we did capture compliance to events in the protocol; for instance, debridement, both the extent and frequency; dressing changes; glucose control; off-loading. And we find that these parameters are well-followed by all centers. And there is no center difference.

In fact, FDA posed a question to us in a fax. And I believe it's in one of the amendments in your briefing booklet. Why is it that you would have seen differences, you know, large differences, between some centers?

Statistically I don't believe that there is a difference. There is a comparability between factors. That hasn't been looked at extensively, but, for instance, Center 2 has really small ulcers and Center 6 did not. Center 2, for instance, had no Charcot patient and the ulcer size.

That's just a notable finding that I think that with the multiple factors of size, anatomical location, Charcot status -- in fact, this slide shows patients with an active Charcot.

Center 2 had no patients with Charcot, 13 Apligraf and 11 control. And Center 5 had three and one. You see that some centers that may not have performed with as high an absolute heal rate in both groups just probably had a distribution of risk factors.

There really could have been an uneven distribution between the centers. I'd ascribe it to a different patient population scene and not to a treatment practice or failure to follow a protocol.

CHAIRMAN WHALEN: Ms. Brinkman?

DR. SABOLINSKI: And ulcer area is actually shown here. I'm sorry. It's just Center 2 is 135. That just struck me as something really small. Center 4 had a median ulcer area of 405, a mean ulcer area of 405. And that's just a -- there are some notable differences.

DR. CHANG: Thank you.

CHAIRMAN WHALEN: Ms. Brinkman?

MS. BRINKMAN: Pass.

CHAIRMAN WHALEN: Ms. Maher?

MS. MAHER: Nothing to add.

CHAIRMAN WHALEN: Dr. McCauley?

DR. McCAULEY: I had several questions. One related to the determination of ulcer size. Is there something specific in terms of your product relative to the maximum size of the ulcer that was allowable in the study?

And part two to that regards: Is there a graph showing the healing of these ulcers as a function of size?

DR. SABOLINSKI: Yes. Let me address the issue of a relationship of ulcer size first. The ulcer size. When I actually showed a slide twice of the factors that we stated prior to beginning the study, ulcer size was one of them. It was input as a continuous variable, which means that it wasn't simply broken out by a particular size. The larger the ulcer -- it was tested in a continuous way.

And in the final model in our

multi-variate analysis, the final Cox model, ulcer size is significant. The larger the ulcer in this study, that is a negative risk factor. Large ulcers heal less well in the overall patient population.

So when you adjust -- and, again, the purpose for the Cox model is to say perhaps -- I mean, we show you a median. We show you a mean for ulcer size group to group. But let's say that size had distributed so that the Apligraf group had smaller ulcers easier to heal. When you adjust for size, how would the overall results have turned out? So adjusting for size, we maintain the difference. But size is a negative prognostic factor.

We just put up the final model where baseline area has a risk ratio of .65 and a 95 percent confidence interval of .48 to .86 with a *p* less than .05.

What that says -- and the confidence interval is actually important, too -- is with a risk ratio less than one, the larger the ulcer, you always heal less well with a larger ulcer. If the confidence interval had included one, it would have said: Well,

sometimes it doesn't make a difference. But an ulcer size does.

DR. McCAULEY: My second question relates to your definition of wound infection. As a surgeon, most of us think of a wound infection from the standpoint of quantitative bacteriology.

If you have greater than 10^5 organisms for gram of tissue, it's probably not amenable to closure. And even in some of these patients where qualitative bacteriology is very important, especially if you find streptococcus, in which you cannot put a skin graft or any type of skin equivalent on the wound.

So I'm kind of curious as to how you define your wound infections. And what do you feel the impact of quantitative bacteriology or qualitative bacteriology would have had on this study, particularly in your failure rates?

DR. SABOLINSKI: Well, first, the evaluation of infection in our study was made on clinical grounds. And we did define in the protocol what this meant, write it out and write it in the case report forms. And this is what it was.

You know the cardinal signs and symptoms, but they include but are not limited to elevated body temperature, cellulitis, streaking, ascending redness, wet gangrene, a purulent odor which is not eradicated with foot cleansing, increased glucose sucosa, greater than 10^5 organism per gram of tissue, or abnormally elevated blood sugars.

We asked that a clinical assessment be made. And we did this because we think that that's the way the product is going to be used.

I think that when -- the publications regarding the quantitative bacteriology showing 10^6 or greater skin grafts don't survive or are unable to take I think was basically evaluated in burn patients and in the acute wound. I'm not aware of a study that's been done.

I think it may have an impact. I think that in this study, for instance, I have been asked by FDA to talk about wound infection. I think it's presumed infection in our study that's being reported or suspected infection. It wasn't required that quantitative bacteriology be done.

And, for instance, in the venous leg ulcer population where no debridement, extensive debridement, was performed, the suspected wound infection rate was reported as being higher. In this study, you were talking about something that occurred in both groups at ten percent or so.

I think that your extensively debriding and having a clean wound base was a positive in this study. And then the infections that are reported I do believe are best described either presumed infection or suspected infection.

DR. McCAULEY: There was a study done by Tom Krizek back somewhere around 1960 in which he looked at patients with open wounds. And all of those wounds that were determined to be clinically uninfected if they were biopsied, 50 percent of those wounds had bacterial counts greater than 10^5.

So you can't use straightforward clinical parameters to tell you whether or not you have a wound infection because a wound can look clean and still have bacterial counts of greater than 10^5.

DR. SABOLINSKI: Yes. And I think that

the performance of a skin construct certainly would be improved if all of the wounds were determined to be of 10^5 or less.

Obtaining biopsies in this patient population and insisting that that be complied with I think is problematic, but I do agree that clinical outcomes would improve with skin graft with less bioburden. I certainly think that would be of help.

CHAIRMAN WHALEN: Dr. DeMets?

DR. DeMETS: Yes. I have a few questions. First of all, going back to your design, as I understand it, you did a randomization and then some screening of the patients. Can you discuss or describe the rationale for that, having it in that sequence?

DR. SABOLINSKI: Yes. The study was initiated in 1995. And the reason why this was done was really purely on practical grounds that in order to get Apligraf to the center, you needed to know who was going to be in which group.

I think in an ideal world, where practical considerations wouldn't have entered in, that it would

have been certainly optimal and more routine to do your randomization at the point of treatment at study day zero.

We didn't do that because we needed to get Apligraf there for those patients who were going to receive it. And, in fact, that posed a potential risk of eliminating patients in that run-in period.

I think that we addressed this in our presentation by showing the comparability of the treated patients. We also did provide information regarding the demographic and the comparability and the reasons for screen failure of the randomized but not treated.

There is a comparability about it. In fact, the demographics of the randomized not treated showed that there were more Charcot patients eliminated from the control group. The wound areas were larger in the control group. You know, the demographics were comparable or maybe the more severe patients and control were eliminated. So when examined, we didn't see an evidence of bias.

DR. DeMETS: You presented some slides

looking at co-variates?

DR. SABOLINSKI: Yes.

DR. DeMETS: Without getting too technical, if you were doing a simple regression model as a co-variate adjustment and you did a correlation coefficient and had an r^2 of, let's say, 40 percent, you could say: Do you explain 40 percent of the outcome by this regression model?

In the analysis that you have done, there is not an exact equivalent of that. But if you're going to argue that you have adjusted away any differences, then you need to come up with some kind of an assessment of how well your model accounts for the outcome.

And there are ways. They're not as simple as an r^2, I must confess, but did you do any of that kind of evaluation in your thinking?

DR. SABOLINSKI: Actually, this is a question that is complex for me. And I'm going to just have to turn around in back of me and ask Jay Herson from Applied Logic.

Applied Logic Associates, Houston, Texas,

performed all of the data management and statistical analyses in this PMA.

DR. HERSON: Yes. Of course, you're right. There are no direct equivalents of an r^2. We did do a goodness-of-fit test for our final model using the log of the minus log, the survival function. And we found that we were not able to reject the hypothesis of a good fit. So the model fits the data according to that goodness-of-fit test.

DR. DeMETS: Okay.

CHAIRMAN WHALEN: Could I just interject? Forgive me for interrupting. Any new speaker, please, is reminded to establish their relationship to the company and whether or not they have financial interests in this or any other device.

DR. HERSON: Right. My name is Jay Herson of Applied Logic Associates. Our company, as Mike said, performed the data management and biostatistics for this clinical trial. Of course, we were paid for those services, compensated, reimbursed for our travel expenses to come to this meeting.

And I don't own any shares of stock in

Organogenesis or any other medical device company.

DR. DeMETS: I've got one final question. In your presentation as well as in your documentation, you comment on the evaluation of the outcome, that you had photographs but did not use them in your analysis. Was there a reason for that?

DR. SABOLINSKI: Yes. The photographs in the study are meant to document some easily assessed parameters. For instance, it does provide documentation. They're basically designed for use in presentation, publication.

It gives us an idea how the groups are doing, but using the Wound Healing Society definition, where not only is epithelium a requirement, full epithelium, but also drainage, we don't find that photographs really allow you to make that determination.

In the past, for instance, in our first study, which both of these studies are not able to be blinded, our skin construct looks quite different from the dressings that observers know, which treatment it is and, in fact, you know is clearly in a photograph,

as you do. So we don't think photographs add.

We have done a correlation for the first submission in the PMA, where we had two blinded observers. And Observer 1 compared an assessment of healing to the case report forms, the investigator assessment; Observer 2 to case report forms; and then, finally, Observer 1 to Observer 2. And the Kappa statistic was greater than .7. There was good agreement between our photos and the investigator assessment.

We find that the more objective data is to make determinations on the basis of tracings so that you can quantitate this and show a progression and then correlate investigator assessments, open tracing assessments. Again, photos are not really able to be used with precision.

DR. DeMETS: One more question? I have one more question. Your statement, your slide which is on Page 89 of your book, which confused you also confused me. Could you try that one more time slower? It has to do with the wound healing as the number of applications go up.

DR. SABOLINSKI: Okay. It's Page 89?

DR. DeMETS: Yes, in your briefing. I don't know which slide it is, but -- yes, that top one. I must say I missed --

DR. SABOLINSKI: Oh, this. The previous slide in your book, on Page 88, shows the data. And this slide was meant to be descriptive of it.

Basically what the words were supposed to convey is that the incidence of adverse events, 184 adverse events occurred in the cohort that had 5 applications. That 184 was 184 over the 345 total adverse events. That incidence is 53 percent.

If we go to the distribution of patients treated in that cohort, 59 patients of the 112 Apligraf patients were in the cohort of 5. That also is 53 percent.

So the next slide. In words, it was attempting to convey that while the number of reported adverse events increases as a function of the number of Apligraf applications, the percent of reported adverse events is very similar to the percent of patients receiving one, two, three, four, or five

applications.

And then this suggests that there's no direct correlation which exists between the number of Apligraf applications and adverse events. I guess the uncompleted thought is that if there were a direct correlation, just by this relationship alone, you would have expected to outstrip the demographic in the population.

For instance, if 90 percent of your adverse events occurred in the cohort that had 5, that to me would be something that would indicate a relationship.

Just the other thing, too. If you go back to the previous slide, this is simply an accounting that the number of adverse events occurred; for instance, 184 events occurred, in those that received 5.

It doesn't say, however, and there is no chronology implied in this slide that the adverse event could have occurred after one or two. It could have occurred at four months or six months. But this was just a mathematical saying, that just the data in

front, seeing this would seem to say that there is no direct causality, number of pieces, and number of adverse events.

CHAIRMAN WHALEN: Dr. Galandiuk?

DR. GALANDIUK: I know that it was not in your exclusion criteria, but what was the percentage of patients with metatarsal ulcers that had previously undergone some type of metatarsal head decompression in the two groups?

DR. SABOLINSKI: I'm going to have to ask for a slide where we show the history of amputations group to group. I don't know the answer off the top of my head for metatarsal head. I know that we did capture these data.

One fact that I do know, in the study we list 7 amputations for patients in Apligraf, 15 in control. Looking at a consensus paper, minor and major amputations, there was only one major amputation in each group.

DR. GALANDIUK: But this wouldn't be an amputation. It would be a metatarsal head decompression --

DR. SABOLINSKI: Correct.

DR. GALANDIUK: -- to help ulcer healing. If you had an unequal distribution, that could significantly skew your results.

DR. SABOLINSKI: The history of amputation of patients who of the 112 and the 96 patients in the study, I don't know the data. They're going to have to pull the slide to show that.

DR. GALANDIUK: That would be just very important I think --

DR. SABOLINSKI: Yes.

DR. GALANDIUK: -- to show between the different groups. Another thing, along with Dr. McCauley, I think the diagnosis of infection in these patients can be very subjective. There was an 11 percent difference in the number of patients who had had antibiotics within the last 30 days --

DR. SABOLINSKI: Right.

DR. GALANDIUK: -- skewed toward more in the Apligraf group. Could that have skewed your later infection simply because the wounds may have been cleaner starting out than the first group?

DR. SABOLINSKI: That's a difficult parameter to assess. We do know that it's not a good idea to prophylax with antibiotics in patients who aren't infected, especially with skin graft or in our skin construct if you're selecting for resistant organisms.

It may have been the explanation you offered. It also could be that the patients were sicker entering into the study. So it could be a bias one way or the other. And I don't think you know.

The history of amputation, again, I don't know if we're able to retrieve metatarsal head amputation, but the number of --

DR. GALANDIUK: Yes. It's not a --

DR. SABOLINSKI: These are the data, 41 and 39 Apligraf to control, who had amputations as a history prior to entering into the study.

DR. GALANDIUK: Although metatarsal head decompression is not an amputation.

DR. SABOLINSKI: Right.

DR. GALANDIUK: So that wouldn't be found in there.

CHAIRMAN WHALEN: Dr. Boykin?

DR. BOYKIN: Yes. Just a few questions, some of which I'll save for a little later. But I'd like to talk a little bit about the design of the study because there's some concern that I have about the comparability of the groups that have been looked at.

We have a pretty exact definition of what complete healing is in terms of epithelization. And as your product is designed, once it is applied to the wound, the wound is 100 percent healed.

What interests me even more are some other preclinical studies that you have in Volume II on Page 2 that review some other issues concerning the viability of the product itself. And this refers to tissue remodeling, cellular persistence in skin construct implants, the characterization of primary and secondary allogenic t-cell responses, remodeling of the construct and the effect of growth factors, and the response of Apligraf to physical injury.

Now, having read this and noting that you have a persistence of viable keratinocytes and

fibroblasts at one year in your experimental model and that human collagen can be found in increased amounts in this experimental model at the same time and that, indeed, this construct behaves with normal phases of repair identical to that seen in human skin, I believe that what you have here is a composite human allograft. I believe that in every sense of the word, this is a skin graft that you have engineered without antigenic factors.

My question is: Should we compare an engineered human skin graft to a wound that's treated with saline-moistened gauze dressings?

DR. SABOLINSKI: Well, my first response is to the last question. We're required in devices not to use a placebo but to compare against standard care. And skin grafting and diabetic foot ulcers is not.

So there are real differences between debridement, saline gauze, and off-loading and an Apligraf treatment. That's something that is unable to be overcome by our requirement to prove effectiveness and safety in comparison to a standard

care approach.

The second I would like to get back to is that you referred to the preclinical tests about persistence remodeling. That is in nude mice. And as a requirement of our approval in 1998, FDA and the sponsor agreed to conduct -- we agreed to conduct a study to demonstrate the longevity of Apligraf cells for its intended use: venous leg ulcer.

It's difficult to do. Ten patients have been enrolled in a ten-patient study. Two of those ten patients have demonstrated Apligraf DNA at week four in our study. Those two patients did not demonstrate Apligraf DNA at week eight.

In the clinical experience in chronic wound, we have no evidence of Apligraf persistence for the life span of the product. And I would suggest that certainly in venous leg ulcer, which is our only experience base to date, it appears that Apligraf does not persist. And, in fact, maybe -- I mean, Dr. Falanga introduced some data regarding how it may behave and can comment about skin grafts.

DR. FALANGA: Yes. I think that it does

not persist clinically. That's been our experience and those who have used this product more extensively as well.

You're right that when you cover a wound surface, by definition you've covered it. And I really didn't want to get into mechanisms of action because they're really unproven. I didn't really want to bring them up at this forum.

It appears to most investigators, including myself, that the mode of action might be one of stimulation of the endogenous repair process. What you have is a construct of viable cells, very dynamic, as I said, and the cells appear to behave in a smart fashion perhaps.

You saw the cytokine profile. I'm not suggesting that *in vivo* that's how it works. We don't know yet. But it might behave that way by stimulating the endogenous wound-healing process because as cells are, after all, smart, they might be able to adapt to the micro environment of the wound.

I really don't think it behaves as a graft, as an autologous graft. And although you cover

the wound with it in the first week or so, it's just not going to persist.

An immunologist well-versed in the behavior of allogeneic constructs appeared to be quite adamant about the fact that it's just not going to persist.

Now, you might be able to pick up perhaps one or two cells by PCR, you know, after several weeks or perhaps even months, but I don't think that that would be clinically relevant.

DR. BOYKIN: So this is your opinion. You really don't have any clinical evidence to back that up, do you? Do you really have any clinical evidence to dispute the experimental studies that show the persistence of the cells of the year?

DR. SABOLINSKI: We have no clinical evidence to show the persistence of Apligraf on chronic wounds beyond four weeks. And, in fact, that occurred at a frequency of ten patients treated to demonstrating this.

Actually, the data -- and we have had it up once or twice now -- on the number of applications,

the fact that you have more patients receiving three, four, and five and that it was restricted to a four-week period is actually just practical evidence that you're not seeing the persistence of the product, even in a short period of time in this patient population.

Just one other comment to the observation that we have a product, a bi-layered construct of epithelium and that you're healed at the point of use. It's a simple point, but in our protocol, you do your evaluation one week post the last and post the last application.

What we find is that of those patients who healed in the Apligraf study, the median time to healing is about 5 weeks, 36 days. And that's just a cohort of the 63 that were evaluated as having complete wound closure.

So I would suggest that it's not an immediate event and it's not something like a skin graft where everybody starts off healed and then all you can do is lose them over time with the failure to take. It doesn't appear to be that picture.

DR. BOYKIN: Well I tend to disagree. Clinically we have not pursued diabetic ulcers as a plastic surgeon because in most instances, the surgical treatment involves very complex cases.

And maybe in this case, you're defining a range of size of ulcer that we should be looking at surgically. Maybe the four centimeter squared ulcer is one that will be healed half the time with an autograft. And that's worth knowing if we're going to look at this particular product.

It's just trying to make sure that we're looking at apples and apples and not apples and oranges. You start out with a wound. You cover it with human epidermis, human dermis, and you keep patching this graft for five weeks. And then we're to look at how it heals.

You can't find the cells. The nude mice studies tell me that they're alive and well, at least in this particular sterile environment. There's got to be some replacement, but you're starting out with a graft. You're starting out with a full composite of human skin. And if you're going to start out that

way, then let's compare it to something that's comparable.

We didn't have this information in '98. I was on that panel. It was a good study. And then perhaps we would have in hindsight done some things maybe slightly differently.

This, you've got a good study here. But I'm just saying that if we're going to look at the cost factors, the quality of life factors, if we're going to say that, yes, this is a reasonable thing to institute in this case, assuming that all of these patients could have had autografts done, then that might have been a reasonable thing to look at.

DR. SABOLINSKI: Well, I think that one of the theoretical benefits of a product that is supplied and it's off the shelf is that perhaps many of the patients who receive the skin construct product would not have been candidates for autografting because of the hospitalization required. They may have been too sick to have a procedure.

I think certainly the only data that we can draw from this study is how it compares to

standard care, which was defined in the protocol. We just don't have any data to support any other comparisons.

CHAIRMAN WHALEN: Dr. Witten?

DR. WITTEN: Yes. I just want to comment that as you go towards your discussion and your vote, the way we would look at it is that the sponsor is not making a claim of an alternative to a skin graft for healing of these ulcers, but they're making a claim that their product is safe and effective for the treatment of full-thickness neuropathic diabetic foot ulcers of greater than two weeks duration with the other descriptors.

So that you want to think about whether or not this product in the study demonstrated that this product can be used safely and effectively for this indication.

And in this case, since they're not making a claim of as an alternative to skin graft but for treatment of diabetic foot ulcers, that's one way that we will appreciate your looking at it when you get to the vote.

DR. BOYKIN: Thank you.

CHAIRMAN WHALEN: Dr. Reger?

DR. REGER: Yes. Thank you very much for a very comprehensive presentation. I have a question that probably requires some clarification in my mind about: How does the graft-treated, healed skin respond to a mechanical loading compared to the non-graft-treated, healed skin?

It may not be that you have had a chance to study this problem, but I think in terms of application, it would be very helpful for me to understand what the response is going to be to the mechanical loads of weight-bearing and shear loads and those that these ulcers would have to withstand after healing.

DR. SABOLINSKI: Well, first, I don't have any tensile strength data. That wasn't captured. It wasn't measured. I think the period of --

DR. REGER: *In vivo*?

DR. SABOLINSKI: *In vivo*. That just hasn't been done. If you're asking if we just take a piece of Apligraf and stretch it, what is it's tensile

strength, I know that that exists.

But I would suggest that that probably is not really relevant for the reason of I believe that what we see in this study is that you have the patient's own skin cells that are resurfacing the wound, certainly out in time.

And I think that how it responds to weight bearing, I don't think we have the answer because this study encouraged off-loading throughout, both in the six weeks of crutches or wheelchairs or using pressure-relieving footwear.

Perhaps the one thing that I can show is just the number of days that ulcers remain closed group to group, which was captured and is certainly limited by a six-month period of observation.

I believe that certainly in -- I don't know if you can bring that slide up. It shows the comparability group to group. What you're seeing of the 36 control patients who were healed and of the 63 Apligraf patients who were being healed, over the entire period of study -- and there is a data listing in our PMA that shows those that complete the study --

it's not very different than these.

Some of these 63 and 36 didn't complete. But the mean number of days in Apligraf is 108 from the day they closed. You know, you get credit for a day of closure if you're observed and 95 in control. And the median is 120 and 103.

So I guess practically we'd suggest that, again, limited by the six months of observation, the strength of the healed skin group to group is comparable.

DR. REGER: There was some mention of reopening of the wounds in the past that I have seen. I don't have exactly in front of me the data.

I'm curious whether that had any relationship to this issue of inadvertent weight bearing. I'm going to call it that or noncompliance or something of that nature.

DR. SABOLINSKI: No. Actually, regarding weight bearing, what we have is documented in our case report forms, compliance as assessed by the investigator of patients and pressure-relieving shoewear.

There was greater than 95 percent compliance at each visit for both groups with *p*'s that were not statistically significant between groups. So our documentation would say that weight bearing is comparable group to group. And then that combined with the finding of in the face of a comparable weight-bearing regimen or off-loading in this case, that the patients who healed performed in this way.

Another slide that gets at the durability of closure or the heal and hold type of response is what percentage of the patients group to group have been closed for greater than or equal to four weeks. And that's over 80 percent in both. So, again, you see a picture of comparability.

I don't know how I can answer the question any better than that. I just don't have any other information.

DR. REGER: May I have one more question? I'd like to follow up on an earlier raised issue of the graft size, wound size, and the relationship of the wound size to the graft size, in particular.

Is there, let's say, an upper limit of the

wound size to the constant diameter of the graft side, I believe? Your graft size is seven and a half centimeters in diameter. So what is the ratio or the average ratio or do you offer some warning as to what's the maximum size wound to treat or anything of that?

DR. SABOLINSKI: I can tell you that there was no consideration of engineering in the size of the ulcers. And the Apligraf that is approved for distribution is approximately three inches in diameter. In fact, both in our venous leg ulcer pivotal trial and here, we know that the graft is cut to fit the wound area.

So, for instance, it may be sectioned so that all of the open area is treated with graft, but it may be one or two units of the three-inch disks that are needed to treat the large ones, you know, really large ulcers.

But we supply only three-inch diameter disks. And the only consideration was to define a range. I would imagine that in a label, that we would certainly be restricted to the range that was studied

in the study, I mean, and prospectively defined. I mean, that would be the only information.

DR. REGER: Do you have some recommendation for the grafts that come from different packages? Will it have to come from the same batch of processed ones or some serial group or something like that?

DR. SABOLINSKI: They do by definition now and would be expected to continue. We didn't cover any of the manufacturing issues, but basically the product is continually produced.

And the lots are made with the same cell bank components. And there is a traceability of our lot to the physician to the physician and back. And both the sponsor and the physician would know when there is a difference in the composition.

I think the recommendation that we would make is that the product is the product. However, in fact, practically you would be using material from the same lot.

Our lots that are available, this is an approximation, but approximately 500 of these units

would be available. So if you exceeded 500, I could see a new lot coming into play. But that, too, would probably be coming from the same donors, from the same material.

DR. REGER: I have a couple of other questions I can ask later on some technical details, for instance.

CHAIRMAN WHALEN: If it's specific to the sponsor, this would probably be the appropriate time to ask that question.

DR. REGER: Thank you. It is specific to the sponsor.

I'd like to know: What is the shelf life of the product? And how do you maintain oxygen concentrations in a living range?

DR. SABOLINSKI: The shelf life of the product is five days from the point of packaging. And Dr. Falanga showed you a picture of the petri dish with the product sitting in it.

DR. REGER: Very impressive.

DR. SABOLINSKI: That petri dish is placed in a plastic bag. And it has a ten percent CO_2

atmosphere in it. And it's sealed. And the shelf --

DR. REGER: Is the other 90 percent oxygen?

DR. SABOLINSKI: The other 90 percent is air.

DR. REGER: Air? So that's not an oxygen?

DR. SABOLINSKI: It's ten percent CO_2, 90 percent atmosphere, air, you know, ambient.

DR. REGER: Oxygen is one-fifth of it.

DR. SABOLINSKI: Right. And at the temperatures of between 20 degrees and 31 degrees Centigrade, the product meets the release specifications for 5 days post the sealing and shipment. And every unit is marked with the expiration date. And clear instructions are not to use beyond expiration.

CHAIRMAN WHALEN: We'll take a ten-minute break and resume with the FDA presentation.

(Whereupon, the foregoing matter went off the record at 3:20 p.m. and went back on the record at 3:34 p.m.)

CHAIRMAN WHALEN: The panel will resume

with the FDA presentation.

DR. DURFOR: Thank you, Dr. Whalen.

Good afternoon. This afternoon, I'd like to introduce the FDA review team that will be discussing the application before you, which is Apligraf for the treatment of neuropathic diabetic foot ulcers.

As you have already heard, the product under consideration is Apligraf, which is a culture skin construct composed of Type I bovine collagen and viable allogeneic human fibroblasts and keratinocyte cells.

This panel previously reviewed the same product in January of 1998 for use on noninfected partial and full-thickness skin ulcers due to the insufficiency of greater than one-month duration and which had not adequately responded to conventional ulcer therapy. The product was approved in May of 1998 for this indication.

Today we will be discussing the use of the product for full-thickness neuropathic diabetic foot ulcers of greater than two weeks duration which extend

through the dermis but without tendon, muscle, capsule, or bone exposure.

The FDA review team and presenters for this application are: myself, Charles Durfor; -- I am the lead reviewer; Dr. Roxi Horbowyj, who did the clinical review; and Ms. Phyllis Silverman, who performed the FDA's statistical analysis of this application.

Dr. Horbowyj?

DR. HORBOWYJ: Hi. I am Dr. Horbowyj, a general critical care surgeon and the clinical reviewer for this application. I will present the FDA clinical perspective on Apligraf as applied to neuropathic diabetic foot ulcers.

I will go over the Apligraf market experience as we know it to date as well as a clinical study of just this design and a closing summary.

Apligraf market experience, as you have heard, Apligraf is a .75-millimeter thick bi-layered construct of cultured human keratinocytes and fibroblasts with Type I bovine collagen.

Langerhans cells as well as melanocytes,

macrophages, and lymphocytes and secondary shelters, such as blood vessels and hair follicles, are not present in the device.

The device is processed aseptically, not dermally sterilized to viable cell counts. And it's packaged in a ten percent CO_2 air, atmosphere, and kept at 20 to 30 degrees C. until use. Its shelf life is about five days plus packing, as the sponsor has described.

Wound infection was the most common adverse event that has been reported, both on the U.S. market and in Canada, with device use for its approved use, which is in chronic venous statis ulcer treatment.

Specifically, in the United States, 14 wound infections have been reported out of 40 adverse events. And this is over 10,000 units sold. In Canada, 4 wound infections were reported out of a total of 14 adverse events and in the sale of over 400 units.

The objective of this study was to determine the safety and effectiveness of Apligraf use

in the treatment of superficial neuropathic diabetic foot ulcers. The target population consisted of relatively healthy, consenting, nonpregnant diabetic adults with full-thickness neuropathic foot ulcers that have no gross evidence of arterial insufficiency or active infection. And these also extended through dermis, but without tendon, muscle, capsule, or bone exposure and without tracts or sinuses associated with them.

The ulcers were to be at least two centimeters away from other ulcers located on the same extremity. And this was to be evaluated post-debridement at both days minus seven; that is, seven days before study day zero.

Ulcers were to be of area one to two centimeters squared post-debridement on study day zero. And they would have responded with less than a 30 percent decrease in size with conservative therapy with study days minus zero, minus seven to zero.

The study was prospective unmasked to be conducted at up to 30 centers. It was actually conducted at 24 centers and had 200 evaluable

patients. It was to demonstrate a 20 percent difference in incidence of closure rate between Apligraf and control-treated patients with 80 percent power and 5 percent significance level.

Randomization, as you have heard, occurred at study day minus seven, one week before this treatment base. Conservative treatment of the study ulcer occurred during that time. And randomized patients were discontinued if inclusion and exclusion criteria were not met at study day zero.

As you have heard, the treatment was a standard protocol for all patients. Specifically, for control, saline-moistened gauze was applied, with Apligraf was applied up to five times in four weeks for those patients who were randomized Apligraf.

For effectiveness, patients were followed for up to three months after study day zero. For safety, patients were followed for six months after study day zero.

Endpoints for safety included laboratory assessments, vital signs, immunologic evaluations, as well as adverse events. Endpoints for effectiveness

included evaluation of median times. Incidence of 100 percent wound closure from study day zero through study week 12 or month 3 and the incidence of 100 percent wound closure by study week 12.

Secondary parameters included recurrence of ulcers or wound characteristics and some unvalidated tools that were designed for the study and included investigator global assessment, a wound assessment, and the treatment-response tool.

Complete wound closure, as you have heard, was defined in this protocol. And the definition was full epithelialization with an absence of drainage as assessed at post-debridement and debridement was necessary.

The wounds were assessed by an unmasked evaluator. Wound tracings were also performed by this unmasked evaluator. But the wound tracings themselves were evaluated by a masked observer.

From the perspective of outcomes, the patient accounting shows that both the treatment and control arms had over 80 percent follow-up at week 12 and mere 80 percent follow-up at 6 months.

There are no remarkable differences between Apligraf and control groups for the solutions of demographics, such as age, gender, race, body mass index, insulin-dependent diabetes, non-insulin-dependent diabetes, smoking history, ulcer size, or location.

From the perspective of effectiveness, tied to the incidence of 100 percent wound closure was found to have a median of 65 days for Apligraf and 90 days for control. This was found to be statistically significant and is clinically significant as well. This is for the overall population.

The incidence of 100 percent wound closure by our own study of week 12 was 56 percent for patients randomized to Apligraf and 38 percent for control, again on the basis of the whole population.

The investigator wound tracing assessment correlation was 0.996, which suggests a very good correlation between investigator wound tracing assessments.

The effectiveness of Apligraf when considered with the number of Apligraf applications

shows that most of the patients who received Apligraf received five applications of Apligraf.

Those who received a single application had the highest incidence of closure for that subgroup. The incidence of closure decreased if you considered incidence per subgroup. However, when you look at the overall population, the incidence of closure increased with applications fairly consistently until the fifth application, where there is a higher incidence of closure.

Base to closure increased with the number of applications, as would be expected. And overall these are the incidences of closure, as discussed on the previous slide.

The sponsor presented outcomes with numerous subgroup evaluations. And they're clinically and statistically significant differences for many of these.

Of note is that in these subgroups, the number of patients is substantial and that the incidences of wound healing are fairly consistent per group in both Apligraf and control, as are the median

times to closure.

There is a slightly longer time to closure for patients with insulin-dependent diabetes. And the incidence there is lower. However, compared to control, this difference is still in favor of Apligraf with only 23 patients in the control healing, as opposed to 49 patients in the Apligraf group.

This is also the case for ulcers that were just single ulcers in a target but associated with Charcot's diseases found in nonsmokers and patients who had good or improving nutrition. Again, these subgroups were substantial. And the trends were consistent per group.

The trends were also consistent; however, without a statistical significance found for patients who were over 70 years old who were smokers who had ulcers in the mid-foot or who had body index greater than the median.

In these cases, however, you see that the subgroups were small. The trends, however, persisted. This subgroup was larger. It's not completely clear why its trend was weaker, but the trend is still